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Antibody Studies of Factor VIII Inhibitor in a Case With Waldenström's Macroglobulinemia

Takeshi Endo,[1] Yutaka Yatomi,[2]* Norihiko Amemiya,[1] Asako Mizoe,[1] Masaki Iino,[3] Hideyuki Ozawa,[4] Shigemi Hosogaya,[1] and Yukio Ozaki[2]

[1]Department of Clinical Laboratory, Yamanashi Medical University Hospital, Yamanashi, Japan
[2]Department of Laboratory Medicine, Yamanashi Medical University, Yamanashi, Japan
[3]Department of Hematology, Yokkaichi Municipal Hospital, Mie, Japan
[4]Department of Internal Medicine, Koshimizu Red Cross Hospital, Hokkaido, Japan

We report a case of Waldenström's macroglobulinemia with prominent bleeding tendency; laboratory investigation revealed an elevated activated partial thromboplastin time. Further laboratory evaluation showed circulating factor VIII anticoagulant, deemed polyclonal IgG, with a titer of 700 Bethesda Units/ml. The factor VIII inactivation kinetics of the patient plasma were identical to those of a type II inhibitor, and the inhibitor was found to recognize the A2 domain of the factor VIII heavy chain. Apparently, paraprotein is not always the cause of reduced activity of coagulation factors in neoplastic dysproteinemias. Am. J. Hematol. 63: 145–148, 2000. © 2000 Wiley-Liss, Inc.

Key words: Waldenström's macroglobulinemia; factor VIII; anticoagulant

INTRODUCTION

Bleeding tendency of varying degrees is often seen in Waldenström's macroglobulinemia [1,2]. The hemostatic abnormalities observed in this dysproteinemia include impaired platelet function, deficiency of blood clotting factors, and excessive fibrinolysis. Thrombocytopenia, hyperviscosity, and local tissue fragility associated with amyloidosis may also contribute to the bleeding diathesis.

In Waldenström's macroglobulinemia, there appears to be reduced activity of one or more of the following coagulation factors: fibrinogen, prothrombin, or factor V, VII, VIII, IX, or X [1,2]. It has been postulated that abnormal monoclonal IgM proteins form a complex with a specific clotting factor so that its function is impaired. Increased clearance of the clotting factor–paraprotein complexes by the reticulo-endothelial system may also occur. It has been reported that paraproteins may act as specific inhibitors of one or more coagulation factors [3,4].

Here we present a case of Waldenström's macroglobulinemia with marked bleeding tendency. We performed antibody studies and found that the presence of anti-factor VIII activity is related to the hemostatic abnormality in this patient. Interestingly, polyclonal IgG, but not monoclonal IgM, in the patient's plasma was responsible for this anticoagulant activity.

CASE REPORT

A 65-year-old man was referred to our hospital for examination of dysproteinemia in December, 1990. The patient had no symptoms at that time. The concentrations of serum total protein and IgM were 8.5 g/dl and 5807 mg/dl, respectively. An immunoelectrophoresis study revealed serum monoclonal IgM with antigenic type λ light chains, and urinary Bence Jones protein (λ type); thus Waldenström's macroglobulinemia was diagnosed. There was no past or family history of bleeding tendency. His condition was stable until June, 1994, when he developed widespread hematoma in the left iliopsoas (demonstrated by computed tomography) and was hospitalized. Physical examination showed numerous mucocuta-

*Correspondence to: Yutaka Yatomi, M.D., Ph.D., Department of Laboratory Medicine, Yamanashi Medical University, Nakakoma, Yamanashi 409-3898, Japan. E-mail: yatomiy@res.yamanashi-med.ac.jp

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TABLE I. Results of Hemostasis Tests

Test	Results	Normal
Platelet count × 10^3/mm^3	357	157–342
Platelet aggregation	Normal	Normal
Prothrombin time (sec)	10.0	9.9
Activated partial thromboplastin time (sec)	85.9	28–45
Fibrinogen (mg/dl)	528	164–337
Antithrombin III (%)	112	70–130
vWF (%)	251	60–170
Coagulation factor (%)		
V	161	50–150
VII	49	65–135
VIII	2	50–150
IX	91	60–140
X	110	56–138
XI	53	65–135
XII	91	50–150
XIII	145	70–140

neous bleeding lesions and mild lymphadenopathy. The patient had no lytic bone lesions.

Laboratory data at admission were as follows. Blood examination showed anemia with 7.3 g/dl Hb; serum protein was 7.5 g/dl, and albumin was 3.6 g/dl. Immunoglobulin quantification showed 1149 mg/dl of IgG, 201 mg/dl of IgA, and 2990 mg/dl of IgM. The results of hemostasis tests are shown in Table I. These tests revealed an abnormality in intrinsic coagulation with a factor VIII level of 2%. Tests of platelet aggregation (induced by epinephrine, ADP, collagen, or ristocetin) were normal.

After admission, the patient was treated with immunosuppressive drugs (corticosteroid and cyclophosphamide), plasma exchange, and supplementation of factor VIII and activated prothrombin complexes. However, the anti-factor VIII titer of this patient remained high despite the treatments, and his bleeding tendency did not improve.

IN VITRO STUDIES

Existence of Factor VIII Inhibitor

The prolonged activated partial thromboplastin time of the patient was not corrected upon mixing with normal plasma in a 1:1 ratio (data not shown). Normal plasma and patient plasma or factor VIII-deficient plasma were mixed in different proportions; following incubation for 2 hr at 37°C, the presence of inhibitor activity was determined by assay of the residual factor VIII activity, which was measured by one-stage procedures using the partial thromboplastin time technique. It was difficult to correct the abnormality in patient plasma, but not factor VIII-deficient plasma, by the addition of normal plasma (Fig. 1). An inhibitor to factor VIII with a titer of 700 Bethesda units/ml was diagnosed [5].

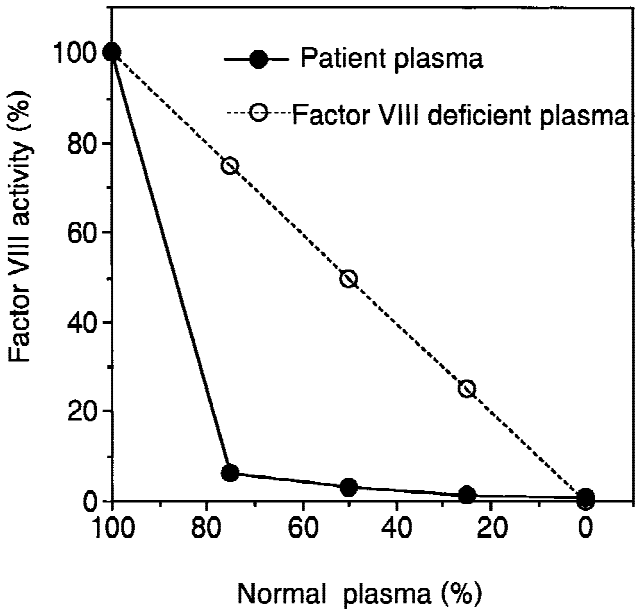

Fig. 1. Failure of normal plasma to correct factor VIII levels in patient plasma. Patient plasma (solid circles) or factor VIII-deficient plasma (open circles) was mixed with normal plasma at the indicated ratios at 37°C, and residual factor VIII activities after a 2-hr incubation were measured.

Kinetics of Factor VIII Inactivation by Patient Plasma

The effect on residual factor VIII activity by varying the patient plasma dilution was observed, as described previously [6,7]. The patient plasma did not completely inhibit factor VIII activity of normal plasma, even when added in excess (Fig. 2); undiluted patient plasma still leave a residual level (1–2%) of factor VIII. Accordingly, the patient inhibitor was considered to be type II [6,7].

Immunoglobulin Classification of Factor VIII Inhibitor Antibody

Diluted patient plasma was mixed with an equal volume of rabbit anti-IgA (α-chain), anti-IgG (γ-chain), anti-IgD (δ-chain), anti-IgM (μ-chain), anti-IgA (α-chain), anti-κ, or anti-λ antiserum (Hoechst, Germany), and left for 2 hr at 37°C. The mixture was then incubated with an equal volume of normal plasma and subjected to assay for residual factor VIII activity. The inhibitor activity was specifically neutralized with anti-IgG (anti-γ) but not with anti-IgM (anti-μ) (Fig. 3). When similarly examined, residual factor VIII activities were 48% and 36% after treatment with anti-IgG1 and anti-IgG4 antiserum, respectively. Furthermore, both anti-κ and anti-λ antiserum were capable of neutralizing anti-factor VIII activity (Fig. 3).

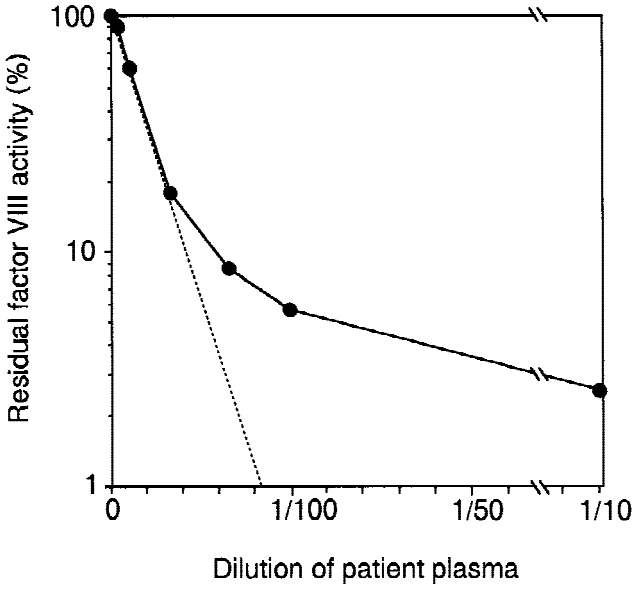

Fig. 2. Mode of action of patient plasma to inhibit factor VIII activity. Patient plasma was diluted at the indicated ratios and then mixed with normal plasma in a 1:1 ratio. After incubation for 2 hr at 37°C, the activity of residual factor VIII was measured and expressed as a percentage of initial factor VIII activity (without patient plasma).

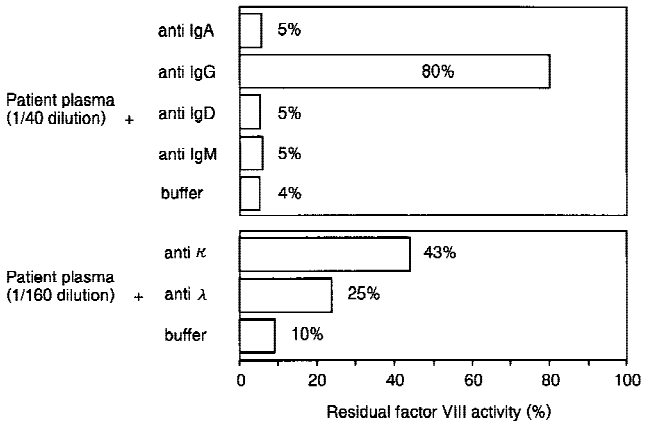

Fig. 3. Immunoglobulin classification of inhibitor antibody. Patient plasma, at the indicated dilutions, was mixed without (control) or with anti-IgA, IgG, IgD, IgM, κ, or λ serum. The mixture was incubated with normal plasma, and residual factor VIII activity was measured.

Determination of the Inhibitor Epitope on the Factor VIII

Since the patient IgG was found to be responsible for anti-factor VIII activity, it was isolated by Protein G-Sepharose (Pharmacia Biotech, Uppsala, Sweden).

The inhibitor epitopes on the factor VIII molecule were determined by immunoblotting with thrombin-proteolyzed recombinant factor VIII (KOGENATE; Miles Inc., USA), as described previously [8]. Mixtures of recombinant factor VIII (77 pmol), thrombin (0.77 pmol), and 5 mM $CaCl_2$ were incubated at 37°C for various durations, and then subjected to 10% SDS–PAGE. After being transferred onto nitrocellulose membranes, the blot was probed with the patient's IgG, and then with peroxidase-conjugated goat anti-human IgG (EY Laboratories, Inc., San Mateo, CA). The reactive bands were visualized by 4-chloro-1-naphthol, *O*-dianisidine, and H_2O_2. As thrombin-induced proteolysis proceeded, the patient IgG was found to react with a 92 kDa, and then 44 kDa, proteolytic fragment of factor VIII (Fig. 4). According to the analysis of factor VIII fragments produced by thrombin proteolysis [8], the patient inhibitor was considered to react with the A2 domain of the factor VIII heavy chain.

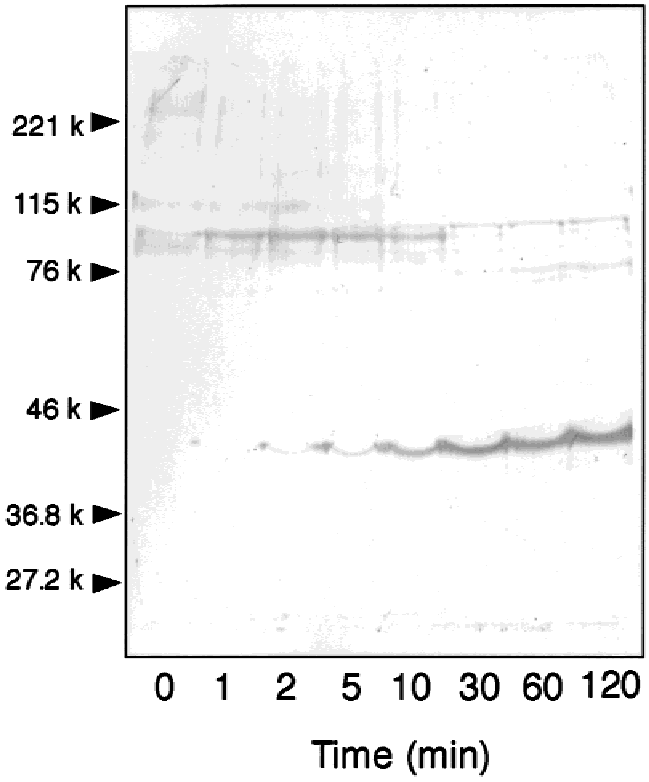

Fig. 4. Localization of the epitope of inhibitor antibody. Factor VIII, degraded by thrombin treatment for the indicated lengths of time, was subjected to 10% SDS–PAGE and transferred to a nitrocellulose membrane. The membrane was then treated with purified patient IgG, followed by peroxidase-conjugated anti-human IgG. The reactive bands were visualized by 4-chloro-1-naphthol, *O*-dianisidine, and H_2O_2.

DISCUSSION

In a previous study of 215 non-hemophilic patients in whom factor VIII autoantibodies developed, persons

aged over 50 years accounted for about one half, with no underlying condition found in many cases [9]. Of those cases in which a clear underlying condition was present, rheumatoid arthritis was the most frequent (7.9%), followed by post-partum (7.3%), malignancy (6.7%), drug-induced cases (5.6%), SLE (5.6%), other autoimmune disorders (4.5%), and dermatologic disorders (4.5%).

In this study, we described a rare case of Waldenström's macroglobulinemia with a circulating factor VIII anticoagulant [9]. Acquired deficiency of factor VIII arising in a patient with macroglobulinemia was previously reported, but the clinical and laboratory findings in that case indicated that the macroglobulin acted as an inhibitor of factor VIII [10]. However, the anti-factor VIII antibody in our case belonged to the IgG class, contrary to our initial expectations. This is consistent with the findings that changes in inhibitor titers did not correlate with those in macroglobulin levels in this case. The patient IgG inhibitor was polyclonal, encompassing IgG1 and IgG4 subclasses, and κ and λ light chains. This is similar to previous reports on inhibition in non-hemophiliacs [11]. The factor VIII inactivation kinetics of plasma from this patient were identical to those of a type II inhibitor, which is also typical of most inhibitors arising in non-hemophiliac patients [6,7].

Human inhibitory alloantibodies and autoantibodies to factor VIII are usually directed at the A2 and/or C2 domains of the factor VIII molecule [8,12]. It was reported that anti-A2 antibodies inhibit activated factor VIII function by blocking the conversion of intrinsic factor tenase–factor X complex to the transition state [12]. The patient inhibitor recognized the A2 domain of the factor VIII heavy chain, which may support the importance of the A2 domain in expressing the cofactor activity of factor VIII.

In summary, the antibody studies of factor VIII inhibitor in this case with Waldenström's macroglobulinemia have indicated that the paraprotein is not always the cause of reduced activity of coagulation factors in neoplastic dysproteinemias. The factor VIII–paraprotein complex may have induced the antigenic recognition by the immune system in this case, although we did not confirm the existence of this complex.

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